the campaign against plague, one expects to find some radical alteration advised; but no, they turn round and join forces, saying, "We recognise that even though a vaccine which is insufficiently standardised, and which is occasionally contaminated, is from the scientific point of view a very imperfect vaccine, yet judged from the standpoint of practical life, such defects may very well be overlooked if the insufficient standardisation and the occasional contamination of the vaccine have not interfered in a sensible manner with its utility.

"This standpoint, which is indeed the only reasonable standpoint, is the standpoint which has been taken up by Mr. Haffkine in the statement that was handed in by him, and which is published, at his request, in our *Proceedings*."

The second part of the report criticises the statistics of anti-plague inoculations. In their summary, the Commissioners say that inoculation diminishes the number of attacks and diminishes the death-rate among those inoculated, that it does not appear to confer any degree of immunity till a few days have elapsed after inoculation, and that the protection lasts certainly for a considerable number of weeks, and possibly for some months.

They recommend that inoculations under safeguards and conditions stated in the report should be encouraged wherever possible, and in particular among disinfecting staffs and attendants of plague hospitals. C. B. S.

EXPERIMENTAL STUDY OF FERTILISATION.

IN 1898 Prof. Yves Delage made a remarkable experiment.[1] He divided the unfertilised egg of a sea-urchin (*Strongylocentrotus lividus*) under the microscope into two parts—one containing the nucleus and the centrosome, the other simply cytoplasmic. Beside these he placed an intact ovum, and then supplied spermatozoa. Towards these the three objects showed equal "sexual attraction"; all three were fertilised; and all three segmented, the intact ovum most rapidly, the nucleated fragment more slowly, the non-nucleated fragment more slowly still. In one experiment, the development proceeded for three days, during which the intact ovum had become a typical gastrula, the nucleated fragment a smaller gastrula, and the non-nucleated fragment a quasi-gastrula with almost no cavity. In each case the cells showed nuclei. The conclusion was then drawn that *fertilisation and some measure of development may occur in a fragment of ovum without nucleus* or ovocentre, and it was suggested that we have in fertilisation to distinguish two processes:—(*a*) the stimulus given to the ovum by a specially energetic kinoplasm brought in by the spermatozoon, perhaps in its centrosome; and (*b*) the mingling of heritable qualities, or amphimixis. One may also note that the experiment was suggestive in furnishing experimental confirmation of what is generally assumed, that each of the sex-cells is a fully equipped potential individuality. Here we may recall the remarkable experiment of H. E. Ziegler,[2] who divided the just fertilised ovum of a sea-urchin in such a way that each half had one pronucleus, and observed that the half with the male pronucleus segmented and formed a blastula.

In 1899 Delage continued his experiments,[3] and with striking success. Non-nucleated fragments of the ova of a species of *Echinus*, of *Dentalium entale*, and of *Lanice conchilega* were effectively fertilised; they proceeded to develop, and gave rise to plutei, veligers, and trochophores respectively. The terms merogonic fertilisation and merogonic development are suggested to express the remarkable facts observed.

The segmentation of the fertilised non-nucleated fragment was practically normal in the sea-urchin, very irregular in *Dentalium*, less irregular in *Lanice* (the chief irregularity being lack of correspondence between the nuclear and the cytoplasmic divisions), but as the development proceeded some self-regulative process reduced the abnormalities to insignificance. The plutei only differed from the normal in the extreme reduction of the arms; the veligers and trochophores were almost typical. They showed no lack of vitality, and although further development did not occur, the same is usually true of normal larvæ reared in similar conditions.

[1] "Embryons sans noyau maternel." *C. R. Ac. Sci. Paris*, cxxvii. (1898), pp. 528–531.
[2] "Arch. Entwickelungsmechanik," iv. (1898), pp. 249–293, 2 plates, 3 figs.
[3] "Études sur la mérogonie." *Arch. Zool. expér.*, vii. (1899), pp. 383–417, 11 figs. See also *C. R. Ac. Sci. Paris*, cxxix. (1899), pp. 645–648.

As to the limits of possible merogony, Delage got some results which are nothing if not striking. A quarter of a *Dentalium*-ovum was fertilised and segmented; about a fifth of a *Lanice*-ovum was successfully treated; but the *chef d'œuvre* of experimental nicety was seen when 1/37 *of a sea-urchin ovum gave rise to an agile blastula*. Delage has christened his pigmy creations—tetartogonic, pemptogonic, &c.—but he seems to hesitate in regard to that arising from the 1/37, for he puts the title "triacosthedomogonique" in a footnote. That there is a limit to merogony he is convinced, but he will not at present fix it. It seems not inappropriate to recall Marchal's description[1] of the strange behaviour of the ovum of *Encyrtus fuscicollis* (one of the parasitic Hymenoptera), which gives rise to a legion of morulæ, and forms a chain of 50–100 embyros within one elongated amniotic envelope. For practical purposes it is convenient to remember that, just as four lancelet embryos may be got by shaking apart the first four blastomeres, so Delage by cutting a sea-urchin ovum obtains three larvæ from one egg.

The issues involved in these experiments are so serious (biologically) that one is naturally led to consider possible criticisms, which Delage in his candid scientific spirit has himself suggested. It is difficult to refrain from the suspicion that there may have been some mistake somewhere. The best criticism would, of course, be to repeat the experiments; but in default of this, let us briefly consider with the author some of the possibilities of error. (*a*) It may be suggested that the eggs experimented with had been previously fertilised by stray spermatozoa; but Delage's experience has been that the spermatozoa die 24–36 hours after liberation; and the water in which the eggs were placed had stood for three or four days in a stone cistern. Moreover, only in one case was segmentation seen among the eggs from which those experimented upon were taken. (*b*) It may be suggested that the segmentation of the fertilised non-nucleated ovum-fragments was not genuine, but a pathological fragmentation such as is occasionally observed after mechanical or chemical stimulation; but it must be remembered that larvæ were reared, and there were, of course, control observations on non-fertilised fragments. (*c*) It may be suggested that the nucleus was cut in the delicate operations, so that each part had really a portion of nucleus as well as cytoplasm. But it must be remembered that the nucleus is very small and very mobile, and thus runs little chance of being cut; in the clear ova of the sea-urchin it was sometimes seen after the operation in the larger part, only once in the smaller part, never in both. In the other two cases (*Dentalium* and *Lanice*) the opacity of the egg hides the nucleus. Perhaps the best answer is, that in one experiment three embryos were got from one ovum, and that fragments representing 1/10 and 1/37 of the total volume of the egg were also seen to segment. It seems, however, possible still to retort that the operation broke the nucleus and caused a distribution of nucleoplasm into the various fragments before they were quite separated.

What are the conditions of successful merogony? Delage failed with the ova of *Ciona*, *Haliotis*, *Chiton*, *Sabellaria*, &c., and he almost failed with those of *Lanice* until he learned the particular "tour de main" in cutting them. The experiment is not practicable except with eggs which are liberated separately before fertilisation. It usually fails if there is a shell. The ova to be tried by other experimenters should be naked or with a delicate glairy envelope, not too brittle, of firm consistence, and not less than 1/10 mm. in diameter. In all Delage's experiments there was a certain percentage of failure, due perhaps to the inability of the fragments to recover from their wounds, or to a diminution in the viscous substance which surrounds the ovum and keeps its parts together. But, in spite of these failures, the number of merogonic segmentations was generally at least equal to, and sometimes greater than, the number of segmentations among intact ova in similar conditions,—a remarkable fact which leads Delage to the daring suggestion that the absence of the female pronucleus may favour fertilisation. "*The female pronucleus is perhaps useful in securing for the embryo the advantages of amphimixis, but it is not useful in fertilisation nor necessary for the development of the parts of the organism.*"

The preceding paragraphs give the gist of Delage's remarkable experiments, but there are some less secure addenda which deserve notice. He has shown the possibility of merogonic hybridisation; he observed phenomena which point to a

[1] *C. R. Ac. Sci. Paris*, cxxxvi. (1898), pp. 662–664. *Ann. Nat. Hist.* ii. pp. 28–30.

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distinction between cytoplasmic and nuclear maturation; he reared a merogonic sea-urchin larva whose cells had the normal number (18) of chromosomes, although the spermatozoon-nucleus (the only one in this case) imported (it is presumed) but half that number. The last fact leads him to conclude that the number of chromosomes is a specific property of the cell.

Although Delage's experiments stand at present alone as regards the method pursued, there have been of late a number of experimental studies on fertilisation, all of which present points of great interest. From among these we select those of Prof. Jacques Loeb,[1] as it seems of particular importance that his results should be collated with those of Delage.

Loeb finds that the mixture of about 50 per cent. of $\frac{10}{8}$ *n* $MgCl_2$ with about 50 per cent. of sea-water is able to bring about (in the eggs of the sea-urchin *Arbacia*) the same result as the entrance of a spermatozoon. After being subjected to this mixture for about two hours, the eggs were returned to normal sea-water, wherein many developed, forming blastulæ, gastrulæ and plutei. Fewer eggs developed than in natural conditions, and the development was slow, but otherwise the results were normal. The author believes that the only reason why the eggs of this sea-urchin and of other marine animals do not usually develop parthenogenetically is the presence or absence of ions of sodium, calcium, potassium and magnesium. The two former require to be reduced, the two latter to be increased.

"*The unfertilised egg of the sea-urchin contains all the essential elements for the production of a perfect pluteus.*" All the spermatozoon *needs* to carry into the egg for the process of fertilisation are ions to supplement the lack of favourable ions, or to counteract the effects of the other class of ions in the sea-water, or both. "The spermatozoon *may*, however, carry in addition a number of enzymes or other material. But *the ions and not the nucleins in the spermatozoon are essential to the process of fertilisation.*"

It is interesting to observe that while Delage's experiments go to show that the nucleus of the sea-urchin ovum is not essential to development, Loeb's experiments go to show that the spermatozoon may (with intact ova) be dispensed with. What is now needed is a combination of the two modes of experiment.

J. A. T.

CHANGES OF COLOUR OF PRAWNS.

IT has long been known that the very numerous varieties of the prawn *Hippolyte* (*Virbius*) *varians* reflect, each after its kind, the colour of the weed or zoophyte to which they cling, and on which they find both food and shelter. A few naturalists, after noting this striking case of "protective resemblance," have detached some of the more brilliantly coloured specimens for the purpose of making a detailed subsequent examination. When they came to do this they found that the vivid brown and other tints had in the interval largely faded, or were replaced by others. This discovery has no doubt been made independently time after time, and has given point and emphasis to the essentially variable character of this prawn. Not only do individuals differ from each other, but any one of them is capable of altering its characteristic tint.

Thus, at the time when Keeble and Gamble began their observations,[2] *Hippolyte varians* was known to change colour, but while one author stated that a sympathetic colour-change was rapidly effected, as well in the dark as in the light, when weed of a new tint was introduced; another affirmed that even in the light the change was slow and did not always agree with the colour of the new weed. Yet a third author stated that darkness by itself has a distinct reddening effect. The only definite conclusion to be drawn from these curiously conflicting statements was that *Hippolyte* offered a fine field for research, and that though a few strollers had here and there plucked an ear or two of corn, there was a fine harvest still to be gathered.

After two years' work on the coasts of Lancashire and of Normandy, Keeble and Gamble have come to the conclusion that three kinds of colour-change may be distinguished in *Hippolyte*.

[1] On the nature of the process of fertilisation and the artificial production of normal larvæ (plutei) from the unfertilised eggs of the sea-urchin. (*Amer. Journ. Physiol.* iii. (1899), pp. 135-138.)

[2] "The Colour-Physiology of *Hippolyte varians*." By F. W. Keeble, Caius College, Cambridge, and F. W. Gamble, Owens College, Manchester. Read before the Royal Society on November 23, 1899.

I. First, a periodic and rhythmic cycle of change composed of a diurnal and a nocturnal phase of colour. Towards evening a decided red tinge—a sunset glow—makes its appearance, and this ushers in the nocturnal change. A green tinge ensues, which spreads fore and aft from the middle of the body. Presently this green colour gives place to an azure-blue colour, which is the characteristic nocturnal tint, and is accompanied by a greatly heightened transparency in the tissues. Under natural conditions this colour-phase persists until daybreak. At the first touch of dawn it disappears, and that of the previous day is gradually reassumed.

More striking even than the distinctive colours is the periodicity of the nocturnal and diurnal phases. Thus, in constant darkness a nocturne (that is a prawn in the nocturnal colour-phase) recovers to its diurnal colour. In constant light, a diurnal form passes over to the nightly phase. Though light often induces, and induces with marvellous rapidity, a recovery from the nocturnal colour to that of the previous day, yet it is often powerless to overcome the habit of the animal. The periodicity is only worn down in the course of two or three days.

It follows that since the colour of *Hippolyte* is a function of the time of day, that time must be taken into account in an investigation on the colours of Crustacea.

II. The second kind of colour-change is the susceptibility of *Hippolyte* to changes of light-intensity. Although the periodic habit of the prawn is the hitherto unknown and yet dominant factor, yet its force is greatest at the times of the assumption of the nocturnal phase, and the resumption of the diurnal tint. At other times external conditions may modify the colour of the animal to a large extent, and the chief agent in the production of these modified colours is the varying amount of light reflected from or scattered by, surrounding objects.

An almost black prawn changed in a few minutes, after being placed in a white porcelain, to a transparent and colourless condition. Further, a ready and almost infallible means of producing green prawns is to place them just after their capture in a white jar, and cover the mouth of the vessel with muslin. Under these circumstances the change—from brown to green, for example—takes place in from thirty seconds to one minute.

Speaking generally, exposure to a low light-intensity during the day favours an expansion of the red pigment, and so produces brown or even reddish effects. Hence, probably the red colour of these prawns at sunset; while an increase in the amount of light, especially if scattered from a white smooth surface, produces a green effect by expanding the blue and yellow pigment and causing the red to contract.

III. The third change differs chiefly from the second in its rate of progress. It is the very slow sympathetic colour-change which ensues when adult prawns, taken from a food-plant of one colour, are placed with the weed of a new colour. Thus, if green *Hippolyte* be placed with brown weed, and the light-intensity maintained unaltered, as far as possible in comparison with the light-conditions of its former habitat, the prawns will retain their green colour even for a week or more, but in the end give way and become brown. Their subsequent recovery when placed with green weed is more rapid. Keeble and Gamble have repeated such experiments time after time in the open, and under as natural conditions as possible, and found that the prawns were either quite refractory or responded in this slow manner. Yet these same specimens, as each evening drew on, underwent the colour-changes culminating in the nocturnal hue with the greatest readiness, and recovered as quickly the next morning to the tint of the previous day.

The great difficulty in ascertaining whether *Hippolyte* responds to change in the colour of its surroundings by a sympathetic change of its own bodily tints is now clear. It lies in their marvellous sensitiveness to changes of light-intensity, as apart from colour, and is increased by the dominant and periodic colour-changes which subvene night and morning. If it were possible to eliminate these two factors, then we might be able to detect the response of *Hippolyte* to colour or change of colour *per se*; in fact, Keeble and Gamble have made an attempt. By the use of colour-screens, based on the instruments used by Landolt and other workers, the prawns are subjected to red, green and blue light, and also a width of a spectrum from the red to the green. The results of these experiments are curious. They show that even when the light transmitted by the screen, and falling on the prawns, is high (the incandescent lamp and a mirror being used to effect this), yet that with red, green and

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